METHODS AND COMPOSITIONS FOR SECRETION OF HETEROLOGOUS POLYPEPTIDES

FIELD OF THE INVENTION

This invention relates to signal sequences for the secretion of heterologous polypeptides from bacteria.

DESCRIPTION OF BACKGROUND AND RELATED ART

Secretion of heterologous polypeptides into the periplasmic space of *E. coli* and other prokaryotes or into their culture media is subject to a variety of parameters. Typically, vectors for secretion of a polypeptide of interest are engineered to position DNA encoding a secretory signal sequence 5' to the DNA encoding the polypeptide of interest. Two major recurring problems plague the secretion of such polypeptides. First, the signal sequence is often incompletely processed or removed, and second, the amount of polypeptide secreted is often low or undetectable. Attempts to overcome these problems fall into three major areas: trying several different signal sequences, mutating the amino acid sequence of the signal sequence, and altering the secretory pathway within the host bacterium.

A number of signal sequences are available for the first approach in overcoming secretion problems. Watson (*Nucleic Acids Research*12: 5145–5164 (1984)) discloses a compilation of signal sequences. U.S. Pat. No. 4,963,495 discloses the expression and secretion of mature eukaryotic protein in the periplasmic space of a host organism using a prokaryotic secretion signal sequence DNA linked at its 3' end to the 5' end of the DNA encoding the mature protein. In particular, the DNA encoding *E. coli* enterotoxin signals, especially STII, are preferred. Chang et al. (*Gene*55:189–196 (1987)) discloses the use of the STII signal sequence to secrete hGH in *E. coli*. Gray et al. (*Gene*39:247–245 (1985)) disclose the use of the natural signal sequence of human growth hormone and the use of the *E. coli* alkaline phosphatase promoter and signal sequence for the secretion of human growth hormone in *E. coli*. Wong et al. (*Gene*68:193–203 (1988)) disclose the secretion of insulin-like growth factor 1 (IGF-1) fused to LamB and OmpF secretion leader sequences in *E. coli*, and the enhancement of processing efficiency of these signal sequences in the presence of a prlA4 mutation. Fujimoto et al. (*J. Biotech.*8:77–86 (1988)) disclose the use of four different *E. coli* enterotoxin signal sequences, STI, STII, LT-A, and LT-B for the secretion of human epidermal growth factor (hEGF) in *E. coli*. Denefle et al. (*Gene*85: 499–510 (1989)) disclose the use of OmpA and PhoA signal peptides for the secretion of mature human interleukin 1β.

Mutagenesis of the signal sequence has, in general, not been especially helpful in overcoming secretion problems. For example, Morioka-Fujimoto et al. (*J. Biol. Chem.*266:1728–1732 (1991)) disclose amino acid changes in the LTA signal sequence that increased the amount of human epidermal growth factor secreted in *E. coli*. Goldstein et al. (*J. Bact.*172:1225–1231 (1990)) disclose amino acid substitution in the hydrophobic region of OmpA effected secretion of nuclease A but not TEM β-lactamase. Matteucci et al. (*Biotech.*4:51–55 (1986)) disclose mutations in the signal sequence of human growth hormone that enhance secretion of hGH. Lehnhardt et al. (*J. Biol. Chem.*262:1716–1719 (1987) disclose the effect of deletion mutations in OmpA signal peptide on secretion of nuclease A and TEM β-lactamase.

Finally, attempts at improving heterologous secretion in *E. coli* by modulating host machinery has so far shown limited improvement in overcoming secretion problems. For example, van Dijl et al. (*Mol. Gen. Genet.*227:40–48 (1991)) disclose the effects of overproduction of the *E. coli* signal peptidase I (SPase I) on the processing of precursors. Klein et al. (*Protein Engineering*5:511–517 (1992) disclose that mutagenesis of the LamB signal sequence had little effect on secretion of bovine somatotropin, and that secretion properties of bovine somatotropin appear to be determined by the mature protein rather than by changes in the signal sequence. Perez-Perez et al. (*Bio/Technology*12:179–180 (1994)) disclose that providing an *E. coli* host with additional copies of prlA4 (secY allele) and secE genes, which encode the major components of the "translocator", i.e., the molecular apparatus that physically moves proteins across the membrane, increased the ratio of mature to precursor hIL-6 from 1.2 to 10.8. U.S. Pat. No. 5,232,840 discloses novel ribosome binding sites useful in enhancing protein production in bacteria through enhanced and/or more efficient translation. U.S. Pat. No. 5,082,783 discloses improved secretion of heterologous proteins by hosts such as yeasts by using promoters of at most intermediate strength with heterologous DNA secretion signal sequences. European Patent Application No. 84308928.5, filed Dec. 19, 1984, discloses promoter-ribosome binding site expression elements of general utility for high level heterologous gene expression.

The instant invention discloses the unexpected result that altered translation initiation regions with reduced translational strength provided essentially complete processing and high levels of secretion of a polypeptide of interest as compared to wild type sequences, and that many mammalian polypeptides require a narrow range of translation levels to achieve maximum secretion. A set of vectors with variant translation initiation regions provides a range of translational strengths for optimizing secretion of a polypeptide of interest.

SUMMARY OF THE INVENTION

One aspect of the invention is a method of optimizing secretion of a heterologous polypeptide of interest in a cell comprising comparing the levels of expression of the polypeptide under control of a set of nucleic acid variants of a translation initiation region, wherein the set of variants represents a range of translational strengths, and determining the optimal translational strength for production of mature polypeptide, wherein the optimal translational strength is less than the translational strength of the wild-type translation initiation region.

In a further aspect of the invention the variants are signal sequence variants, especially variants of the STII signal sequence.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. **1** depicts the sequence of the PhoA promoter, Trp and STII Shine-Dalgarno regions and STII signal sequence.

FIG. **2** is a diagram depicting relevant features of the plasmid pLS33.

FIG. **3** is a diagram depicting construction of the library, pSTIIBK.

FIG. **4** is a graph depicting comparison of the levels of expression of IGF-1, as measured by the amount of IGF-1 detected in culture supernatants, for pLS33, pSTIIBK#131, and pSTIIC. Experiments 1 to 8 represent measurements taken on 8 separate dates.